The Influence of Diet Composition on the Availability of Various Phosphorus Sources

By

Robert Bruce Christmas

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Robert Bruce Christmas

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A series of trials was conducted to evaluate the utilization of various types of chick diets in the assay of phosphorus-containing materials, as well as to determine possible metabolic complementary effects of combinations of phosphorus sources. Broiler-type chicks were used in all trials except those which involved a purified diet. A total of six different basal diets was used in these studies and all trials were of two or three weeks' duration. Chicks were housed in wire floored, electrically heated, battery-type brooders with feed and water supplied _ad libitum_. Tibia ash, body weight and mortality rate were the evaluation criteria.

Mortality was extremely high in all treatments when purified diets were fed, but no statistically significant treatment differences were noted. Bone ash favored treatments which received sodium acid phosphate over those which received soft phosphate. Additional phosphorus from calcium phytate did not improve performance. Due to high mortality in all groups, purified diets were not ideal for the assay of phosphorus sources in these experiments.

Graded levels of soft phosphate or calcium phosphate gave a linear response in tibia ash and body weight when evaluated in a degerminated

corn meal diet. A similar response in tibia ash was noted when graded levels of calcium phosphate were added to a hominy feed diet. This graded response was not noted in bone ash with soft phosphate nor body weight with both sources unless calcium:phosphorus ratios were expanded. The addition of low levels of soft phosphate to both diets gave a marked response in bone ash. Good body weight and poor bone ash were obtained with a 0.50 percent, all plant phosphorus hominy feed diet. A combination of soft phosphate and plant phosphorus with 0.71 percent total phosphorus gave optimum body weight. Additional studies with hominy feed diets confirmed that calcium:phosphorus ratios were critical in determining the availability of phosphorus, and that comparisons should be made with consideration of these ratios. Good bone ash levels were obtained with soft phosphate and good growth with plant phosphorus. To some extent, there appeared to be a complementary effect between the two. Utilization of phosphorus in soft phosphate appeared to be quite high at low levels of supplementation, but decreased as the supplemental level increased. A hominy feed diet may be useful in phosphorus assays, since growth rate is equal for all supplemental phosphorus groups, thus removing this factor from consideration.

The addition of 3.0 or 6.0 percent fish meal numerically improved bone ash in soft phosphate supplemented practical corn-soybean type broiler chick diets. Some improvement in body weight was noted when 6.0 percent fish meal was added. Soft phosphate and calcium phosphate gave equal performance in a 6.0 percent fish meal diet. Equal performance was also obtained whether phosphorus came from calcium phosphate or fish meal when in combination with soft phosphate. These data indicated that fish meal made no dietary contributions which could not be replaced with

calcium phosphate and other nutrient sources.

Even though statistically significant differences could not be noted in many instances in these trials, soft phosphate did not consistently perform as well as other sources evaluated. Why soft phosphate appeared to be highly available at low levels is not completely understood. There still appears to be specific roles for different forms of phosphorus in body metabolism.

CHAPTER 1

Introduction and Literature Review

The infinite purpose of agricultural research is to feed, clothe and care for the greatest number of people in the most economical and best possible manner. Research in animal nutriton has attempted diligently to carry out this purpose and consequently has initiated and conducted the study of many nutrients. One of these, phosphorus, has probably been involved in more investigations than any of the others. This is not necessarily because phosphorus is any more important than the others, but because it is an integral and critical part of so many key functions in plant and animal life. This element plays a vital role in mineralization functions for the formation of bone, teeth and egg shell. It is also extremely important in many metabolic processes. It carries out important functions in carbohydrate, lipid, protein, vitamin and inorganic salt metabolism. It is involved in lactation. It is a vital part of one of the most important reactions known to man - - the assimilation, storage and release of energy in the living cell. With its involvement in nucleic acids, it is an integral part of each body chromosome. It is an important part of many body buffer systems. Body structure and life itself are dependent upon the animal obtaining an adequate and usable supply of phosphorus. Of course, no less could be said about countless other nutrients. Phosphorus, however, appears to be a major factor in many of these life processes and, therefore, a dependable source of phosphorus is directly and

indirectly an important need.

Over 300 years have transpired since Brandt, a German chemist, first prepared phosphorus in a free state. It was not until some 100 years later in 1769 that Gahn, a Swedish chemist, found that it was an essential part of animal bone. Only 110 years ago, according to Ewing (1963), did phosphorus supplementation begin to be a part of animal nutrition. Van Gohren corrected weak bones in cattle grazing certain areas near the Rhine river by feeding them small amounts of bone meal. Grass and soil in the area were later found to be low in phosphorus.

There are many sources of phosphorus available to supply domestic livestock needs. The first and most common source is from the forage and feedstuffs consumed by the animal. This is seldom adequate within itself and requires additional supplementation of concentrated amendments. There has been considerable research and opinion on availability of plant phosphorus. Phosphorus extracted from plants was found to be poorly available in rat and chick studies by Lowe et al. (1939), Krieger et al. (1940, 1941), Spitzer et al. (1948), Gillis et al. (1949, 1957) and Matterson et al. (1946). Others report different degrees of availability for plant phosphorus sources varying from slightly available to readily available. A large part of the phosphorus contained in plant materials is in the form of phytic acid (inositol hexaphosphoric acid) and its salts. Peeler (1970) reported that phosphorus in phytic acid was quite available under certain conditions but phosphorus in calcium phytate (one of the acid salts) was relatively unavailable. Many nutritionists feel that only approximately 30.0 percent of the phosphorus in plant sources is available.

Other important sources of phosphorus include the more concentrated ones, such as bone meal, rock phosphate, calcium phosphate and soft phosphate. It was believed for many years that only phosphorus from organic sources (plant and animal) was available to animals; consequently, this resulted in the delay in use of mineral stores. Fluorine toxicity further complicated the widespread use of rock phosphate. However, defluorination processes made the use of rock phosphate common, beginning some 30 years ago. In recent years, the treatment of lime with phosphoric acid has been perfected to yield calcium phosphate, a high quality, relatively fluorine-free product which is high in phosphorus and calcium. Colloidal phosphate (soft phosphate) has also become quite available in recent years. This product is obtained from sediment areas created in the mining and washing process of rock phosphate. It is relatively inexpensive and could contribute greatly to the animal industry if it could be adapted to commercial animal diets; however, considerable work has indicated that the phosphorus in this material is not as available as that in the more commonly used sources. There are many other sources of phosphorus which for one reason or another have not found common usage.

Several assay methods and procedures are utilized in determining phosphorus availability. These assays may be chemical in nature or may involve measured responses of life processes of experimental animals. These response measurements included X-ray, phosphorus deposition, bone ash, growth rate, bone breaking strength and others. There is considerable variability among the phosphorus availability values determined by these procedures and measurements. For example, Damron (1968) found that soft phosphate was quite available when bone ash was the evaluation criterion, but not very available when growth was the criterion. Other researchers

have found similar results. Consequently, much research has been conducted to determine a good assay procedure; however, there is still no one concise procedure and method.

Numerous studies have been conducted to establish the phosphorus requirement for starting chickens. These vary from the 0.38 to 0.47 percent suggested by Singsen _et al_. (1947) to a 0.76 to 0.81 percent level reported by Couch _et al_. (1937). McGinnis _et al_. (1944), Singsen _et al_. (1948), Gillis _et al_. (1949), O'Rourke _et al_. (1952), Grau and Zweigart (1953), Fisher _et al_. (1953) and Nelson and Walker (1964) have suggested levels in between these two extremes. Again these levels vary with material used, response criteria, and type of chicks used. The National Research Council (1971) has established the minimum dietary phosphorus requirement at 0.70 percent with at least 0.50 percent of this total from inorganic sources. Approximately 30.0 percent of the phosphorus of plant products is non-phytin phosphorus and can be considered as part of the inorganic phosphorus requirement. However, these levels are affected by many factors including the interaction of calcium and phosphorus, calcium:phosphorus ratios, the level of vitamin D and type of chicks.

Extensive evaluation has been conducted to determine the relative availabilities of both organic and inorganic phosphorus. Several methods have been utilized in these processes. Gillis _et al_. (1954) proposed that bone ash could be used as a criterion in phosphorus assay and this has become an accepted means of evaluation. Growth rate is also considered an important evaluation criterion.

Ideally, a diet that can be completely controlled in all nutritional aspects would be desirable. Gillis _et al_. (1954) used a purified diet

in their studies. They found that this diet supplemented with soft phosphate would not support life in chick trials. The diet was originally designed to study the potassium requirement of the chick. Wilcox et al. (1953) modified this diet to study the phosphorus and calcium requirement of turkey poults; however, forage or buttermilk had to be added to support maximum growth. The attempted evaluation of soft phosphate with this purified diet resulted in the mortality of all poults by the nineteenth day of the trial. Many researchers feel that the disadvantages of a purified diet are greater than the advantages. It is felt that the results obtained with this type diet cannot be applied to practical conditions. With these reasons in mind, a phosphorus assay diet utilizing degerminated corn meal was developed by Vandepopuliere et al. (1961) at the Florida Station. The basal diet contained 0.30 percent phosphorus which was low enough to allow graded levels of the test material to produce a linear response in bone ash and growth.

The utilization of phosphorus in soft phosphate has been investigated by many workers in the past few years. The general consensus of many of these has been that soft phosphate was not utilized to a large degree by the chick. Waldroup et al. (1965a) reported that approximately 55.0 percent of the phosphorus in soft phosphate was available. Summers et al. (1959) found its availability to be 47.0 percent. Nelson and Peeler (1961) reported a biological value of only 34.0 percent for the phosphorus in soft phosphate. However, Damron and Harms (1968a) found little difference in bone ash at 21 days between chicks receiving comparable levels of soft phosphate and calcium phosphate. Differences observed in weight were not statistically significant. Later studies by Damron and Harms (1968b) yielded similar

results. Baruah et al. (1960a), using bone ash as a criterion, indicated that soft phosphate was quite available. Another report from Baruah et al. (1960b) confirmed these results but stated that adequate growth could not be obtained with soft phosphate alone. Growth rate obtained was greater when one-fourth of the added phosphorus from soft phosphate in combination with calcium phosphate or defluorinated phosphate was compared with an equal amount of sodium acid phosphate. These results indicated that multiple assay procedures might be needed when determining the availability of phosphorus in a particular source material. These data may also suggest that the phosphorus in soft phosphate may be available for mineralization processes but not for growth. The possibility exists that the growth and mineralization processes may require, or at least may be able to utilize, phosphorus in different forms. Waldroup (1965), using a degerminated corn meal chick diet, found that low levels of soft phosphate consistently produced a higher percent bone ash than did comparable levels of calcium phosphate. Later studies by Waldroup et al. (1969) found no difference in bone ash when soft phosphate was compared to calcium phosphate. There were no significant differences in growth and feed efficiency when rates were comparable. While attempting to establish a standard calcium curve, Damron and Harms (1969a) found that soft phosphate gave bone ash levels, but not body weight, equal to those obtained with calcium phosphate or defluorinated phosphate. Summers et al. (1959) reported that mixing soft phosphate with phosphoric or hydrochloric acid increased its availability. Waldroup et al. (1965c), attempting a similar study, noted that the acidulation of soft phosphate with phosphoric acid yielded a product higher in phosphorus than expected.

They attributed this largely to the loss of water vapor during the chemical reaction which occurred while mixing. The combination improved both body weight and bone ash over that of soft phosphate alone; however, the authors reported that the acidulated products supported no greater performance than was expected from the amount of phosphorus supplied by the various ingredients.

There have been several experiments to determine the cause of the low availability of the phosphorus in soft phosphate. In their review Motzok et al. (1956) suggest that the poor performance of soft phosphate is due to the extensive levels of metaphosphate and pyrophosphate forms present. This does not explain, however, the reason for the degree of mineralization obtained with soft phosphate. Damron and Harms (1971) studied the availability of phosphorus in calcium metaphosphate and calcium pyrophosphate. They observed that calcium:phosphorus ratios were important in determining availability. Calcium metaphosphate was not nearly as available as monosodium phosphate. Calcium pyrophosphate generally did not support performance comparable to that of monosodium phosphate; however, at certain levels of supplementation differences were not statistically significant. Watts and Miner (1959) suggested that the calcium in soft phosphate was much less available to the chick than calcium from other sources. Harms et al. (1961) postulated that this was the reason for poor performance in the diet of laying hens.

Bethke et al. (1928) and Hart et al. (1930) reported that calcium: phosphorus ratios were very important in the response to phosphorus and calcium by growing chicks. These ratios were shown to be especially critical with soft phosphate (Motzok et al., 1967; Fritz et al., 1969;

and Waldroup, 1965). Even though ratios are important there has been considerable variation in assay ratios. Creech et al. (1956) and Nelson and Peeler (1961) maintained a constant calcium:phosphorus ratio in experimental diets. Ammerman et al. (1960) used a constant 1.0 percent calcium and Gardner et al. (1959) used a constant 1.2 percent. Waldroup et al. (1965a) reported that variations in procedures used in phosphorus assays could influence the relative biological value of the phosphorus source. From this work, it was suggested that for maximum performance a different ratio or level of calcium might be used for each level of phosphorus evaluated. Therefore, Harms et al. (1967) and Damron and Harms (1968b, 1969) conducted a series of experiments to establish the best calcium level (or ratio) for each respective phosphorus level fed. These studies involved several different phosphorus sources including sodium acid phosphate, calcium phosphate, defluorinated phosphate, soft phosphate and Curacao Island phosphate. Standard curves were developed for each phosphate material.

The performance of chicks fed diets containing soft phosphate were shown by Damron et al. (1967) to be improved by the addition of 3.0 percent fish meal. These workers attributed this improvement to a readily available source of phosphate. However, in subsequent studies Damron and Harms (1968d) found that an equal amount of phosphorus from defluorinated phosphate did not result in maximum growth. Damron and Harms (1969) evaluated the addition of magnesium to diets supplemented with soft phosphate and monosodium phosphate. High levels of magnesium depressed performance at all levels of soft phosphate supplementation and all but one level with supplemental monosodium phosphate.

Plant phosphorus is considered by many nutritionists to be relatively

unavailable; however, Waldroup et al. (1965b) reported that near optimum weight gains in chicks could be obtained from a hominy feed diet which contained 0.5 percent total phosphorus, all of which came from plant sources. However, maximum mineralization could not be obtained from this diet. These weight gains were superior to those obtained from a degerminated corn diet which had a comparable total level of phosphorus, but with 0.2 percent from inorganic sources. The phosphorus supplemented degerminated corn diet in Waldroup's trial (Waldroup et al., 1965b) was far superior for bone mineralization to the 0.5 percent all-plant phosphorus level. Mineralization tended to increase as the level of inorganic phosphorus increased regardless of the basal diet. These data indicate that plant phosphorus is available for growth but not adequate for optimum mineralization. Fritz et al. (1947), Vandepopuliere et al. (1961) and Waldroup et al. (1965b) reported similar findings. In contrast, the literature reviewed indicated that soft phosphate might be available for mineralization but not for growth.

There were several purposes for conducting the investigations reported in this dissertation. First there was a need to determine if the phosphorus in the economical and available soft phosphate would cause death or not support chick life as had been indicated by previous trials with purified diets. Second, phosphorus appears to play two major roles in body function. Research suggests that certain forms or configurations of phosphorus might perform one role but not the other and that the reverse might be true for other sources. Trials were conducted to determine if the phosphorus from soft phosphate was selectively available for mineralization but not for optimum growth,

as well as to determine if the inverse of this situation were true for plant phosphorus. Third, the comparative evaluation of degerminated corn meal diets and hominy feed diets for phosphorus assay purposes appeared to be desirable. Finally, the need to determine if plant phosphorus or soft phosphate utilization could be enhanced by combining with other phosphorus supplement sources such as mineral stores or fish meal was apparent. If such information could be determined, a greater dimension in phosphorus supplementation could be realized.

CHAPTER 2

Materials and Methods

Chicks involved in the experiments reported in this paper were placed in electrically heated battery brooders with raised wire floors at one day of age. Dietary treatments and water were supplied *ad libitum* for the duration of each test period. On the final day of each experiment all birds from each replicate group were separated and group weighed according to sex. Where available, a representative sample of birds in each replicate group were sacrificed and the left tibia removed and identified for ash determination. Bone ash procedures as described for the A.O.A.C. (1965) method of vitamin D analysis were used in these studies. The bones were boiled in water for four minutes, cleaned of adhering tissue, and lightly polished with cheesecloth. After drying for 24 hours at 100°C., the bones were alcohol extracted for 24 hours, ether extracted for 24 hours, redried, and then individually ashed at 600°C. for six hours. The data were subjected to the analysis of variance procedures outlined by Snedecor and Cochran (1967). Duncan's multiple range test (1955) was used to determine significant differences among treatment means.

CHAPTER 3

Evaluation of Soft Phosphate in Purified Diets

An ideal diet for determining the availability of a phosphorus source, or any other nutrient, is one that is palatable, and one which contains life and growth supporting levels of all known essential nutrients except the one being assayed. The diet should be sufficiently low in the assayed nutrient to yield a distinguishable response to graded additions of the material assayed. A purified diet with these attributes would eliminate the nutrient variability of feedstuffs and give the researcher more complete control of his experimental diet.

Gillis et al. (1954) used a purified diet which contained about 0.03 to 0.05 percent phosphorus to study the availability of various phosphorus sources to the chick. These workers reported that this diet, when supplemented with various levels of soft phosphate and fed to Single Comb White Leghorn cockerels, would not support life. They, however, did not report levels of mortality within these or other treatment groups. Nelson and Peeler (1961) fed a similar diet to Single Comb White Leghorn cockerels and reported that all chicks fed the unsupplemented basal diet died within ten days. They agreed that this diet supplemented with soft phosphate would not support life. They found that 0.15 percent beta-tricalcium phosphate (the reference phosphate used) had to be included in the diet in order

to assay soft phosphate. Using this modified diet, an availability of 34.0 percent was reported for soft phosphate. These workers reported similar findings with a practical diet and suggested that results obtained with purified diets could be applied to practical conditions.

These experiments were conducted to evaluate soft phosphate in a purified diet, as well as to study the effect of calcium:phosphorus ratios on the performance of this material.

Experimental Procedure

Since Andrews *et al*. (1971) determined that there were definite phosphorus source response differences between Single Comb White Leghorn cockerels and broiler-type chicks, Single Comb White Leghorn cockerels were used in these experiments. Mortality was very high in all treatments; therefore, all three experiments were terminated at 14 days and tibia ash and mortality were the only criteria used in evaluation.

The basal diet, formulated as near as possible to that described by Gillis *et al*. (1954), contained 0.07 percent phosphorus and 0.06 percent calcium by analysis. The composition of the diet is shown in Table 1. This is also similar to the diet used by Nelson and Peeler (1961). Soft phosphate (9.70 percent phosphorus and 18.24 percent calcium) and sodium acid phosphate (22.45 percent phosphorus) were used as phosphorus sources. Calcium phytate was an added source of calcium and phosphorus in experiment 3. Reagent grade calcium carbonate was added to each treatment where necessary to obtain the desired calcium levels.

Table 1. Composition of purified diet

Ingredient	Percent of diet
Dried blood fibrin	20.0
Gelatin	4.0
Hydrogenated vegetable fat	3.0
Ground cellophane	3.0
Liver fraction "L"	1.0
Corn starch	65.0
Micro-ingredients[1]	4.0

[1]Supplies per kilogram of feed: 12.00 mg. vitamin A (250,000 I.U. per gm.), 131.00 mg. vitamin D_3 (3,000 I.C.U. per gm.), 13.20 mg. thiamine, 13.20 mg. riboflavin, 22.00 mg. calcium pantothenate, 6.60 mg. pyrodoxine, 26.40 mg. niacin, 4.40 mg. folic acid, 0.40 mg. biotin, 0.022 mg. vitamin B_{12}, 110.00 mg. para-amino benzoic acid, 2.20 mg. vitamin K (menadione), 1.10 gm. inositol, 1.98 gm. choline chloride, 44.00 mg. alpha-tocopherol concentrate, 2.64 gm. $MgSO_4$, 0.26 gm. $MnSO_4 \cdot 4H_2O$, 4.99 gm. iodized NaCl, 110.00 mg. $FeSO_4 \cdot 7H_2O$, 11.00 mg. $CuSO_4 \cdot 5H_2O$, 11.00 mg. $ZnCl_2$, 11.00 mg. $CoCl_2 \cdot 6H_2O$, and 4.40 gm. KCl.

Routine brooding and evaluation procedures were presented in Chapter 2 of this dissertation. Due to the extreme variability of the data obtained, the three experiments were analyzed and discussed separately.

Experiment 1

Eight Single Comb White Leghorn cockerels were randomly assigned to each of three replicate groups per treatment. The seven dietary treatments (Table 2) were: the unsupplemented basal, two graded levels of sodium acid phosphate, and four groups containing two graded levels of soft phosphate with two calcium levels each. Two or more chicks per replicate were sacrificed for tibia ash determination. Both bone ash and mortality data were subjected to statistical analysis, as outlined in Chapter 2 of this dissertation.

Experiment 2

The procedure followed in this experiment was identical to that in experiment 1 except that a commercial broiler diet was added as a positive control treatment. Bone ash and mortality data were subjected to statistical analysis as described in Chapter 2 of this dissertation.

Experiment 3

Eight Single Comb White Leghorn cockerels were randomly assigned to each of three replicate groups per treatment, with a total of 15 dietary treatments (Table 2). The first eight treatments of this experiment were identical to those of experiment 2. Six more treatments were identical to the soft phosphate and sodium acid phosphate treatments except that they contained an additional 0.10 percent phosphorus and

Table 2. Mortality of chicks fed various levels of phosphorus and calcium supplied from soft phosphate, sodium acid phosphate and reagent grade calcium carbonate (3 experiments, 24 chicks per treatment)

Supplemental Phosphorus Source	Suppl. P (%)	Total P (%)	Total Ca (%)	Mortality (%)[1]			
				Exp. 1	Exp. 2	Exp. 3	Exp. 3 Plus Phytate[2]
None	----	0.07	0.06	91.7[a]	66.7[bc]	58.3[bcd]	20.8[ab]
Soft phosphate	0.20	0.27	0.42	87.5[a]	66.7[bc]	66.7[cd]	62.5[cd]
			0.55	83.3[a]	58.3[b]	66.7[cd]	58.3[bcd]
	0.30	0.37	0.57	75.0[a]	70.8[bc]	83.3[d]	62.5[cd]
			0.73	95.8[a]	66.7[bc]	45.8[bcd]	41.7[bc]
Sodium acid phosphate	0.20	0.27	0.55	54.2[a]	58.3[b]	33.3[abc]	33.3[abc]
	0.30	0.37	0.80	83.3[a]	87.5[c]	25.0[abc]	37.5[abc]
Commercial broiler diet	----	0.70	1.00	----	0[a]	0[a]	----

[1]Statistical analysis performed within experiment only. Means within experiment with no common letter are significantly different according to Duncan's multiple range test ($P < 0.05$).

[2]All treatments in last column received 0.10 percent phosphorus and 0.055 percent calcium from calcium phytate, except the first treatment in the column which received 0.20 percent phosphorus and 0.11 percent calcium from calcium phytate. The phosphorus and calcium from calcium phytate were supplemented in addition to that listed in this table.

0.055 percent calcium from calcium phytate. One added treatment, identical to the unsupplemented basal treatment in the first half of the trial, received 0.20 percent phosphorus and 0.11 percent calcium from calcium phytate. Data were collected and analyzed as described in previous experiments.

Results and Discussion

Experiment 1

Mortality was extremely high in this experiment (Table 2). By the fourteenth day, mortality was at least 80.0 percent in six of the seven treatment groups. The lowest mortality was 55.0 percent in the group receiving the sodium acid phosphate supplemented diet with 0.20 percent phosphorus and 0.55 percent calcium. There were no statistically significant differences among mortality of any of the treatment groups in this experiment.

The addition of soft phosphate to the basal diet did not improve bone mineralization at the lower phosphorus level (Table 3). The addition of 0.30 percent soft phosphate phosphorus was not beneficial at the lower calcium level but did increase bone ash at the higher calcium level. The addition of both levels of sodium acid phosphate significantly improved bone ash over the basal and three of four treatments which received soft phosphate. It appeared that there was inadequate phosphorus at all treatment levels to support life, or that there was some other limiting factor in the basal diet.

Table 3. Tibia ash of chicks fed various levels of phosphorus and calcium supplied from soft phosphate, sodium acid phosphate and reagent grade calcium carbonate (3 experiments)

Supplemental Phosphorus Source	Suppl. P (%)	Total P (%)	Total Ca (%)	Tibia ash (%)[1]			
				Exp. 1	Exp. 2	Exp. 3	Exp. 3 Plus Phytate[2]
None	----	0.07	0.06	24.5[ab]	21.7[a]	27.4[ab]	27.4[a]
Soft phosphate	0.20	0.27	0.42	24.5[ab]	22.4[a]	27.1[ab]	34.9[bcd]
			0.55	26.9[bc]	28.3[c]	31.4[b]	31.0[b]
	0.30	0.37	0.57	21.3[a]	24.0[ab]	19.5[a]	29.8[ab]
			0.73	29.3[cd]	27.0[bc]	31.4[b]	29.6[ab]
Sodium acid phosphate	0.20	0.27	0.55	32.1[de]	34.4[d]	44.5[d]	33.5[bc]
	0.30	0.37	0.80	34.6[e]	36.1[d]	34.7[bcd]	42.9[cd]
Commercial broiler diet	----	0.70	1.00	----	42.2[e]	43.3[cd]	----

[1]Statistical analysis performed within experiment only. Means within experiment with different letters are significantly different according to Duncan's multiple range test ($P < 0.05$).

[2]All treatments in last column received 0.10 percent phosphorus and 0.055 percent calcium from calcium phytate, except the first treatment in the column which received 0.20 percent phosphorus and 0.11 percent calcium from calcium phytate. The phosphorus and calcium from calcium phytate were supplemented in addition to that listed in this table.

Experiment 2

The results of this experiment (Tables 2 and 3) were quite similar to those obtained in experiment 1. Mortality was almost 70.0 percent for the total experiment by the fourteenth day. There was little difference in mortality regardless of treatment when chicks were fed the purified diet. When the trial was terminated, the treatment which received the commercial broiler diet had experienced no mortality. Again, soft phosphate gave no improvement in bone ash over the basal diet when the low calcium levels were fed. Increasing calcium levels in the soft phosphate treatments resulted in increased bone ash. Sodium acid phosphate gave an increase in bone ash over all other treatments receiving purified diets. Bone ash for the treatment receiving the commercial broiler diet was significantly greater than all other treatments.

Experiment 3

The addition of 0.10 percent phosphorus from calcium phytate to the diet did not improve bone ash nor livability. Even though mortality was quite high in this experiment, livability tended to favor those treatments which received supplemental phosphorus from sodium acid phosphate. There appeared to be no improvement in livability when treatments were supplemented with soft phosphate. Again, the treatment which received the commercial broiler diet experienced no mortality. As in the previous trials, the addition of soft phosphate to the basal diet did not significantly improve bone ash. The addition of sodium acid phosphate increased bone ash numerically if not significantly over that obtained with basal and

soft phosphate supplemented diets. The treatment supplemented with sodium acid phosphate to a phosphorus level of 0.37 percent produced a bone ash value equivalent to the one fed the commercial broiler diet. The bone ash data collected from the purified dietary treatments in this experiment were extremely variable within any given treatment. Therefore, a large difference in bone ash was required to indicate statistical significance. The addition of the calcium phytate failed to improve mortality or bone ash. This would indicate that the low level of phytin phosphorus was not the factor responsible for the poor performance of the chicken.

Summary

Three experiments using Single Comb White Leghorn cockerels were conducted to evaluate the availability of soft phosphate in purified diets. The data obtained were very variable both within and among treatment groups. Mortality was extremely high when purified diets were fed in all three experiments regardless of phosphorus treatment. There appeared to be no difference in mortality due to treatment; however, in the third experiment, livability favored those treatments supplemented with sodium acid phosphate. A positive control treatment in each of two experiments experienced no mortality during the 14-day experimental period. The addition of soft phosphate to the basal diet failed to consistently increase bone ash. The addition of sodium acid phosphate, however, yielded a numerical if not statistically significant increase over the basal and the soft phosphate supplemented treatments. The inclusion of added phosphorus from calcium phytate did not improve livability nor bone ash.

The data in these experiments indicate that phosphorus in soft phosphate cannot be utilized with this diet nor can that from sodium acid phosphate when livability is the criterion. It is questionable whether this diet is adequate to assay phosphorus sources under the conditions of these experiments. Even though sodium acid phosphate gave an increase in bone ash over the basal diet and the soft phosphate supplemented diet, mortality with the diet was such that practical interpretation was difficult. It is felt that a practical diet would be best to assay phosphorus sources in that mortality would not be a major contributing factor.

CHAPTER 4

Evaluation of Two Dietary Levels of Plant Phosphorus for Use in Phosphorus Assays

Plant phosphorus has been reported to be low in biological availability for the chick and the rat by Lowe et al. (1939), Krieger et al. (1940, 1941), Spitzer et al. (1948), Gillis et al. (1949, 1957) and Matterson et al. (1946). However, Waldroup et al. (1965b) found that optimum weight gains, but not maximum mineralization, could be obtained from a hominy feed diet which contained 0.5 percent phosphorus, all coming from plant sources. Fritz et al. (1947), Vandepopuliere et al. (1961) and Sieburth et al. (1952) reported similar findings. Work by Heuser et al. (1943) and McGinnis et al. (1944) noted limited availability of phosphorus from plant sources. Gillis et al. (1949) suggested that natural plant phosphorus was more effective than that extracted as calcium phytate. Results cited by Peeler (1970) reflected similar findings. Singsen et al. (1947) and Boutwell et al. (1946) found a positive relationship between plant phosphorus utilization and the presence of vitamin D.

The phosphorus in soft phosphate has been found to be low in availability by numerous workers as previously cited. However, an equally large number of researchers have reported adequate to good bone mineralization from diets evaluating soft phosphate. Damron and Harms (1968a, 1969a), using a degerminated corn meal diet, observed the same manifestation. Even though several researchers reviewed by

Motzok et al. (1956) suggested that the poor performance of soft phosphate was due to a large amount of metaphosphate and pyrophosphate present, this still does not answer the question as to why normal, or almost normal, bone ash can be obtained with soft phosphate.

From a review of published research, it appeared that soft phosphate might be utilizable for mineralization while plant phosphorus might be available for growth. These experiments were designed to determine if there might be specific functions which these two phosphorus sources could selectively supply in a complementary fashion as well as to contrast the degerminated corn meal and hominy feed assay diets.

Experimental Procedure

In each of two experiments five male and five female broiler-type chicks (Peterson X Peterson) were randomly assigned to three replicate groups receiving each of 30 dietary treatments. General procedures are described in the materials and methods section of this paper. Four birds in each replicate (two of each sex) were sacrificed for bone ash determination.

The two basal diets involved in this study are shown in Table 4. Both diets contained 22.0 percent protein, 2200 I.C.U. of vitamin D_3, and 2712 kilocalories of metabolizable energy per kilogram of diet. The degerminated corn meal basal was identical to the one used by Damron and Harms (1969a) and was found by analysis to contain 0.30 percent phosphorus and 0.26 percent calcium. The hominy feed basal was similar to one used by Waldroup et al. (1965b) and contained 0.50 percent phosphorus and 0.21 percent calcium. Supplemental phosphorus was supplied from commercial grade soft phosphate (9.70 percent

Table 4. Composition of degerminated corn meal and hominy feed diets.

Ingredient	Diet 1 (Degerminated corn) (%)	Diet 2 (Hominy feed) (%)
Degerminated corn	51.7	----
Hominy feed	----	39.2
Cerelose	5.4	13.8
Corn oil	----	4.5
Soybean meal (50% protein)	34.0	34.0
Alfalfa meal (20% protein)	3.0	3.0
Iodized salt	0.4	0.4
Micro-ingredients[1]	0.5	0.5
Variable[2]	5.0	4.6
% phosphorus	0.30	0.50
% calcium	0.26	0.21

[1]Supplied per kilogram of diet: 7,000 I.U. vitamin A, 2,200 I.C.U. vitamin D_3, 550 mg. choline chloride, 40 mg. niacin, 4.4 mg. riboflavin, 13.2 mg. pantothenic acid, 22 mcg. vitamin B_{12}, 125 mg. ethoxyquin, 20 mg. iron, 2 mg. copper, 200 mcg. cobalt, 1.1 mg. iodine, 100 mcg. zinc, and 83.6 mg. manganese.

[2]Composed of calcium and phosphorus sources and builders' sand.

phosphorus and 18.24 percent calcium) and calcium phosphate (21.69 percent phosphorus and 20.91 percent calcium) in both experiments. Reagent grade calcium carbonate was used to obtain the desired calcium levels in each treatment.

Table 5 outlines the dietary treatments evaluated. Six treatments utilized the degerminated corn meal basal which was supplemented with soft phosphate and calcium phosphate. The three levels of phosphorus and calcium fed with each of the two supplemental phosphorus groups were selected from a test performance standard curve proposed by Damron and Harms (1969). The remaining 24 treatments in each experiment involved the supplementation of a hominy feed basal diet with both soft phosphate and calcium phosphate to total phosphorus levels of 0.57, 0.64 and 0.71 percent. Three levels of calcium were fed within each of the three soft phosphate phosphorus levels.

Since the experiments were identical and statistical evaluation of the data revealed no significant treatment X experiment or treatment X sex interaction, the experiments were combined for publication.

Results and Discussion

When the degerminated corn meal basal (0.30 percent phosphorus) was fed, a graded response was obtained in tibia ash and body weights from supplemental levels of 0.07, 0.14 and 0.21 percent phosphorus from either source. Also, there was no significant difference in bone ash of chicks fed the two supplements at either of the two low levels (0.07 and 0.14 percent) of either source (Table 5). Calcium phosphate was significantly ($P < 0.05$) more available for mineralization than

Table 5. Tibia ash and body weight of chicks fed various levels of phosphorus and calcium supplied from soft phosphate and calcium phosphate in degerminated corn basal and hominy feed basal diets

Supplemental Phosphorus Source	Suppl. P (%)	Total P (%)	Total Ca (%)	Tibia ash[1] (%)	Body weights[1] (gms)
<u>Degerminated corn basal diet</u>					
Calcium phosphate	0.07	0.37	0.47	36.2^{de}	327^{ghijkl}
	0.14	0.44	0.58	39.6^{ijkl}	330^{hijklm}
	0.21	0.51	0.69	42.1^{no}	360^{o}
Soft phosphate	0.07	0.37	0.56	37.1^{defgh}	283^{ab}
	0.14	0.44	0.62	37.7^{efghi}	298^{bcd}
	0.21	0.51	0.68	39.9^{jklm}	310^{defg}
<u>Hominy feed basal diet</u>					
None	----	0.50	0.57	35.5^{cd}	321^{efghij}
			0.67	33.5^{b}	306^{cdef}
			0.77	31.1^{a}	273^{a}
Calcium phosphate	0.07	0.57	0.67	37.9^{efghi}	344^{lmno}
			0.77	35.8^{cd}	315^{defghi}
			0.87	36.7^{defg}	318^{efghij}
	0.14	0.64	0.77	40.7^{lmn}	354^{no}
			0.87	40.0^{jklm}	340^{klmn}
			0.97	38.3^{ghij}	322^{no}
	0.21	0.71	0.87	42.1^{no}	347^{mno}
			0.97	41.6^{mno}	342^{klmn}
			1.07	43.0^{o}	353^{no}
Soft phosphate	0.07	0.57	0.57	39.1^{ijkl}	335^{jklm}
			0.67	38.2^{fghij}	328^{ghijkl}
			0.77	35.7^{cd}	311^{defg}
			0.87	34.0^{b}	283^{ab}
	0.14	0.64	0.67	39.6^{ijkl}	332^{ijklm}
			0.77	38.6^{hijk}	320^{efghij}
			0.87	37.7^{efghi}	305^{cde}
			0.97	36.4^{def}	292^{bc}
	0.21	0.71	0.77	40.3^{klm}	344^{lmno}
			0.87	40.3^{klm}	330^{ijklm}
			0.97	38.7^{hijk}	324^{fghij}
			1.07	38.4^{ghij}	321^{efghi}

[1]Means with different superscripts are significantly different according to Duncan's multiple range test ($P < 0.05$).

was a comparable level of soft phosphate when supplemented at the level of 0.21 percent phosphorus (total 0.51 percent). Body weight data significantly ($P < 0.05$) favored calcium phosphate over soft phosphate at all three levels of supplementation in the degerminated corn meal basal diet. These results generally agree with those published by Damron and Harms (1968a). However, these data reflect considerably more mineralization with the low level of soft phosphate supplementation than these workers reported. Body weight was depressed at all levels of soft phosphate supplementaiton of the degerminated corn meal basal, but the trend as reported by Damron and Harms (1968a) for a decrease in weight as the level of soft phosphate increased was not noted with this particular diet.

Chicks fed the unsupplemented hominy feed grew well but had inadequate bone mineralization. These results agree with those reported by Waldroup et al. (1965b). When this diet was supplemented with 0.07 percent phosphorus from either source, good growth was obtained. With the unsupplemented hominy feed diet containing 0.50 percent total phosphorus, highest mineralization and body weight levels were obtained with a calcium level of 0.57 percent. As calcium increased up through 0.67 percent to 0.77 percent, a progressive depression in both body weight and mineralization was apparent. This trend was also observed when either calcium phosphate or soft phosphate was supplemented with calcium at increased levels (Table 5). As the calcium:phosphorus ratio widened a depression of body weight and mineralization occurred. An exception to this trend occurred when the diet was supplemented with 0.21 percent phosphorus from calcium phosphate. A linear response of tibia ash was obtained from adding each level of calcium phosphate to

the hominy feed diet.

When the soft phosphate supplemented ratio of calcium:phosphorus was approximately 1:1, regardless of level evaluated, mineralization and body weight approached levels equal to those of calcium phosphate. Any expansion of this ratio gave a marked decline in bone ash and body weight. This condition became less critical when adequate levels of phosphorus were present, regardless of the source. These data substantiate the fact that calcium levels are extremely important when the phosphorus levels are sub-optimum. These ratios appear to be more critical for soft phosphate supplemented diets.

Optimum mineralization and body weights were obtained when the degerminated corn meal diet was supplemented with calcium phosphate and calcium carbonate to levels of 0.51 percent phosphorus and 0.69 percent calcium. A hominy diet supplemented to 0.57 percent phosphorus and 0.70 percent calcium from calcium phosphate gave optimum body weight but sub-optimum bone ash. It appeared that the plant phosphorus was available for growth but not for bone mineralization.

The availability of low levels of soft phosphate with narrow calcium:phosphorus ratios is demonstrated by the following observation. The unsupplemented hominy feed basal (total plant phosphorus 0.50 percent) gave an average bone ash of 35.5 percent and body weight of 321 gms. The addition of 0.07 percent phosphorus from soft phosphate yielded a bone ash value of 39.1 percent (significantly higher) and an average body weight of 335 gms. (not significantly different). This increase in bone ash and lack of increased growth might have been expected since bone ash was greatly depressed with the basal diet and growth was not. Also, it may be observed that 0.37 percent phosphorus in the soft phosphate supplemented

degerminated corn meal basal gave numerically higher bone ash values than did 0.50 percent of all plant phosphorus from an unsupplemented hominy feed basal diet. Body weight in this comparison significantly favored the unsupplemented hominy feed basal.

These data appear to substantiate the claim that soft phosphate is available for mineralization but not as readily utilized for body weight gain. Also, the statement that plant phosphorus is more available for growth than for mineralization is supported by these results. The complementary effect observed when soft phosphate was used with hominy feed diets appeared to be extremely sensitive to levels of phosphorus supplementation and Ca:P ratios. It appears that phosphorus in soft phosphate was quite available at low levels of supplementation in the hominy feed diet and that this availability decreased with increased phosphorus supplementation. The fact that the lower calcium level in each of the lower levels of phosphorus supplementation consistently gave the best performance as evaluated by bone ash suggested that the calcium might have been utilized from the phytin phosphorus molecule, thus making phosphorus more available.

Why soft phosphate appears to be relatively available for mineralization and not for growth is not understood, nor is the reverse of this, involving plant phosphorus. It seems that there might be a specific chemical configuration that soft phosphate cannot supply for growth and a similar situation where organic phosphorus cannot be utilized for optimum mineralization. Additional study and research may give more information on the specific causes for these anomalies.

Summary

Two experiments were conducted to evaluate diets with two dietary levels of plant phosphorus for use in phosphorus assays. It was attempted to determine if a hominy feed diet supplying organic phosphorus would be complementary to soft phosphate supplementation for growth and mineralization. A degerminated corn meal diet was used to evaluate mineralization, availability and growth inability with supplementation of soft phosphate.

These data indicate that, with certain qualifications, soft phosphate may be quite available for mineralization but not growth. A linear response was obtained in tibia ash and body weights from feeding supplemental levels of 0.07, 0.14 and 0.21 percent phosphorus from either source when the degerminated corn meal diet was used. A linear response of tibia ash was also obtained from adding each level of calcium phosphate to the hominy feed diet. An increase in bone ash was obtained from supplementing this diet with soft phosphate; however, it was not linear as found for calcium phosphate. It would appear that a hominy feed diet may be useful in phosphorus assays since growth rate is equal for all groups containing supplemental phosphorus, thus removing this factor from consideration in phosphorus availability. It also appears that under certain conditions soft phosphate and organic phosphate might be used complementally to obtain optimum body weight and bone ash.

CHAPTER 5

Further Studies on a Hominy Feed Basal for Use in Phosphorus Assays

The availability of phosphorus in soft phosphate to the chick has undergone extensive evaluation in recent years. Generally, the concensus has been that soft phosphate was not utilized to a large degree by the chick. The findings by many have indicated that this lack of availability was reflected in growth response, but not necesarily in bone mineralization. Many workers have reported that adequate mineralization could be obtained with soft phosphate. Damron and Harms (1968a) found little difference in bone mineralization of 21-day old chicks which had received comparable levels of soft phosphate and calcium phosphate in a degerminated corn meal diet. Another study by Damron and Harms (1969a) yielded similar results.

Waldroup et al. (1963) found that calcium:phosphorus ratios were more critical in soft phosphate supplemented diets than in diets supplemented with phosphorus sources considered more readily available. Motzok et al. (1967) also found ratios to be extremely critical when soft phosphate was employed as a dietary supplement. Research findings discussed in the previous chapter of this paper indicated that calcium: phosphorus ratios were critical in hominy feed diets supplemented with both soft phosphate and calcium phosphate. Ratios appeared to be even more important than phosphorus levels beyond the 0.5 percent plant phosphorus inherently present in the diet. Since a graded response

was noted in bone ash, it might be possible that a hominy feed diet could be utilized in phosphorus availability trials. Body weight could be eliminated since it would not be an experimental variable under these conditions.

The following experiments were conducted to compare sources of phosphorus at various levels of calcium supplementation and to investigate possible complementary effects of these sources. Further evaluation of the effects of reduced calcium:phosphorus ratios was also made.

Experimental Procedure

In each of two experiments, five male and five female broiler-type chicks were randomly assigned to three replicate groups receiving each of 24 dietary treatments for a 21-day period. Shaver X Hubbard chicks were used in the first experiment; Peterson X Peterson chicks were used in the second. Routine brooding and evaluation procedures were described in the materials and methods chapter of this paper. On the final day of each experiment four birds in each replicate group (two of each sex) were sacrificed for tibia ash determinations. The composition of the hominy feed basal diet (Table 4) was similar to the one used by Waldroup *et al*. (1965a) and identical to the one used in the experiments described in Chapter 4. This diet contained 22.0 percent protein and 2712 kilocalories of metabolizable energy per kilogram of feed. The basal diet also contained 0.50 percent phosphorus and 0.21 percent calcium by analysis. A total of 2200 I.C.U. of vitamin D_3 per kilogram of diet as well as other recommended levels of micro-ingredients were supplemented. Table 6 outlines the dietary

Table 6. Tibia ash and body weight of chicks fed various levels of phosphorus and calcium supplied by soft phosphate and calcium phosphate in a hominy feed basal diet

Supplemental Phosphorus Source	Suppl. P (%)	Total P (%)	Total Ca (%)	Tibia ash[1] (%)	Body weights[1] (gms)
None	----	0.50	0.37	37.8[de]	366[d]
			0.47	39.8[f]	366[d]
			0.57	39.9[fg]	378[def]
			0.67	36.7[cd]	345[c]
Calcium phosphate	0.07	0.57	0.57	41.5[ghijk]	388[ef]
			0.67	42.0[ijk]	388[ef]
	0.14	0.64	0.67	42.9[jkl]	393[f]
			0.77	43.1[kl]	371[de]
	0.21	0.71	0.87	45.0[m]	379[def]
			0.97	44.4[lm]	383[def]
Soft phosphate	0.07	0.57	0.37	33.5[a]	320[b]
			0.47	37.8[de]	372[def]
			0.57	41.1[fghij]	377[def]
			0.67	41.4[ghijk]	375[def]
			0.87	37.6[de]	340[c]
			0.87[2]	34.4[ab]	302[b]
			0.97	35.5[bc]	304[b]
			0.97[2]	33.5[a]	272[a]
	0.14	0.64	0.57	39.3[ef]	375[def]
			0.67	41.8[ijk]	387[def]
			0.77	40.9[fghi]	373[def]
	0.21	0.71	0.67	39.9[fgh]	372[def]
			0.77	41.7[hijk]	373[def]
			0.87	42.3[ijk]	376[def]

[1]Means with different superscripts are significantly different according to Duncan's multiple range test ($P < 0.05$).

[2]Supplemental calcium supplied by reagent grade calcium sulfate. Supplemental calcium in all other treatments supplied by reagent grade calcium carbonate.

treatments evaluated. Supplemental phosphorus was supplied from commercial grade soft phosphate (9.70 percent phosphorus and 18.24 percent calcium) and calcium phosphate (21.69 percent phosphorus and 20.92 percent calcium) in both experiments. Desired calcium levels were obtained by supplementing with reagent grade calcium carbonate or reagent grade calcium sulfate. Four levels of calcium were fed to chicks which received the basal diet without phosphorus supplementation. Three graduated increments of 0.07 percent phosphorus were obtained by supplementing with calcium phosphate. Two levels of calcium were fed within each of these increments. Soft phosphate was also supplemented to attain total phosphorus levels of 0.57, 0.64, and 0.71 percent. Six levels of calcium were fed within the first phosphorus level. Two additional calcium levels were fed utilizing calcium sulfate as the supplemental calcium source. It was postulated that calcium sulfate might assist in the utilization of soft phosphate since its solubility is lower than that of calcium carbonate. Three levels of calcium were fed with each of the other two levels of phosphorus supplied by soft phosphate. The lowest levels of calcium within each phosphorus supplemented group were formulated to yield a more narrow calcium:phosphorus ratio that was investigated in Chapter 4 of this paper.

Since both experiments were conducted under identical conditions, and there was no treatment X sex interaction, data were combined for analysis and discussion.

Results and Discussion

The hominy feed basal which contained 0.50 percent phosphorus, all from plant sources, and supplemented to a level of 0.57 percent calcium, yielded body weights which were not statistically different

from the best obtained in the trial (Table 6). The bone ash value of the best phosphorus unsupplemented treatment was significantly lower than the maximum bone ash levels obtained. The growth obtained with this basal diet indicated that the chick could utilize plant phosphorus from a hominy feed diet for growth. The basal diet with 0.37 percent calcium supported good growth; however, bone ash was significantly lower than for higher calcium. This would indicate that the plant calcium as well as plant phosphorus is preferentially used for growth rather than bone mineralization.

When calcium:phosphorus ratios were comparable, the addition of 0.07 percent phosphorus from either source produced an increase in bone ash. This was significant with calcium phosphate and approached significance with soft phosphate. The findings reported in Chapter 4 showed a significant increase in bone ash with the addition of 0.07 percent phosphorus from either source. Calcium phosphate supplementation of 0.07 percent phosphorus gave a ten-gram increase in body weight over the unsupplemented basal. The addition of 0.07 percent phosphorus from soft phosphate did not give an increase in body weight. However, the addition of phosphorus in graduated increments of 0.07 percent from either source gave a numerical increase in bone ash in a linear fashion when calcium levels were optimum.

A bone ash value of 45.0 percent was obtained when calcium phosphate was supplemented to a level of 0.21 percent phosphorus with a level of 0.87 percent calcium. This value was significantly greater than all other values observed except that from a treatment supplemented with 0.21 percent phosphorus from the same source with 0.97 percent calcium. Soft phosphate supplemented at a comparable level and ratio

yielded 42.3 percent bone ash. As noted in previous work, soft phosphate appeared to be utilized to a greater extent for mineralization at lower supplemental levels. Deviation from optimum calcium: phosphorus ratios resulted in a marked depression of bone ash and body weight when soft phosphate was supplied at the three graduated increments of 0.07 percent phosphorus.

There were no significant differences in body weights among treatments receiving the various levels of phosphorus supplementation from either source as long as calcium:phosphorus ratios were optimum and/or comparable. The hominy feed diet supplemented with soft phosphate gave body weights which were not significantly different from those obtained with calcium phosphate. Where levels of calcium adequate to permit observations were evaluated, deviation from the optimum calcium:phosphorus ratio depressed body weight and bone ash. The fact that there was little difference in body weight among phosphorus levels indicated that bone ash could be considered the major evaluation criterion when a hominy feed basal was used.

There appeared to be some complementary effect from combining the phosphorus from soft phosphate and that from hominy feed since 387-gram treatment weight averages were obtained using this combination.

It was postulated that calcium sulfate might make the high level of soft phosphate more available. The result was exactly the opposite. Those treatments which received the calcium sulfate as a supplemental calcium source had significantly poorer bone ash and body weights than did those treatments with identical levels of phosphorus and calcium which utilized calcium carbonate as a supplemental calcium source. The cause of these results was not evident.

Even though differences in body weight of comparable treatments were not statistically significant, groups receiving soft phosphate did not perform quite as well as did those which received calcium phosphate. Calcium:phosphorus ratios appeared to be much more critical when soft phosphate was utilized as the supplemental phosphorus source.

The findings of these trials were similar to those reported in Chapter 4. In these trials a further reduction of the calcium: phosphorus ratios failed to increase bone ash or body weight regardless of phosphorus source or level. Maximum bone ash and body weight within each phosphorus level was obtained when the calcium:phosphorus ratio was approximately 1.2:1.

Summary

The most narrow calcium:phosphorus ratios (approximately 1.2:1) which were evaluated in the previous chapter of this paper gave maximum performance in this trial within each phosphorus treatment group regardless of criteria used or source evaluated. A further reduction of this ratio resulted in decreased or equal performance within all phosphorus treatment groups regardless of phosphorus source. These data indicated that comparisons in phosphorus assays must be made within or with consideration of ratios. As the level of phosphorus considered optimum was approached, the effect of adverse calcium:phosphorus ratios became less apparent.

A hominy feed basal containing 0.50 percent phosphorus, all from plant sources, gave body weights which, even though numerically lower, were not statistically different from those obtained with phosphorus

levels considered optimum in chick diets. Bone ash values from these phosphorus unsupplemented hominy feed treatments were considerably lower than those from diets considered optimum in phosphorus content. This indicated that phosphorus in the hominy feed basal may be quite available for chick growth but not quite so available for mineralization.

Little difference was noted in body weights among the graduated phosphorus treatments as long as calcium:phosphorus ratios were comparable. With the same treatments, the graduated increase in phosphorus resulted in a numerically linear increase in bone ash regardless of source. This suggested that when hominy feed diets are employed for phosphorus assay purposes, body weight data could be eliminated as an experimental variable.

Percent utilization of soft phosphate for mineralization appeared to be greater when supplemented at lower levels and optimum calcium: phosphorus ratios. Increased supplemental levels of soft phosphate appeared to result in decreasing efficiency of utilization of phosphorus in soft phosphate when compared to calcium phosphate under similar conditions.

Although soft phosphate was not statistically inferior, calcium phosphate consistently had better performance. Ratios and levels appeared to be more critical with soft phosphate utilization than with calcium phosphate utilization.

CHAPTER 6

Utilization of Phosphorus Sources With and Without Fish Meal in Practical Corn-Soybean Type Broiler Diets

Many attempts have been made to improve or enhance the utilization of soft phosphate in chick diets. Combination with other phosphorus sources has been one of the most common procedures. Johnson et al. (1953) combined 2.0 percent soft phosphate with 0.5 percent bone meal and obtained good growth and calcification. This diet was superior to one with 1.0 percent soft phosphate and 0.5 percent bone meal, indicating that soft phosphate was being utilized. Waldroup et al. (1965c) obtained improvement in the performance of soft phosphate supplemented diets when either calcium phosphate, phosphoric acid or sodium acid phosphate was added. Soft phosphate plus either of these three performed better than comparable diets with 0.1 percent less phosphorus and no soft phosphate. Summers et al. (1959) obtained an increased availability of the phosphorus in soft phosphate when it was combined with phosphoric or hydrochloric acid. However, Waldroup et al. (1965c) reported no additive effect of two ingredients. These workers noted an increase in the phosphorus content of the treated soft phosphate, and concluded that this increase was due to loss of water vapor in the mixing process. Motzok et al. (1965), Fritz and Roberts (1966), and McKnight and Watts (1966) found that, under certain conditions, vitamin D_3 in excess of that normally supplemented could improve the performance of soft phosphate. Waldroup et al. (1963)

found that when phosphorus levels were sub-optimal, the calcium: phosphorus ratio was more critical in diets containing soft phosphate. The findings discussed in Chapters 4 and 5 markedly support this point. Damron et al. (1967) evaluated soft phosphate supplemented corn-soybean meal type diets with and without fish meal. Graduated levels of calcium were included in the study. These researchers noted that maximum body weights could not be produced by diets containing soft phosphate as the sole supplemental phosphorus source. Body weights favored those treatments receiving fish meal in all but one instance when soft phosphate was the supplemental phosphorus source. There were no differences in bone ash due to fish meal supplementation between treatments with comparable levels of phosphorus. The differences in body weight between the control diet with or without fish meal were not statistically significant when phosphorus levels were comparable. The fish meal dietary treatments containing 0.40 percent phosphorus from soft phosphate were statistically equal to the control group at eight weeks of age. The authors attributed the improved response of birds receiving fish meal diets to the high availability of the phosphorus supplied by the fish meal.

The experiments reported in this chapter were designed to evaluate the utilization and adequacy of soft phosphate and calcium phosphate with and without fish meal in the chick diet. Efforts were made to determine whether soft phosphate would suffice as a phosphorus source when fish meal was present in the diet.

Experimental Procedure

In each of two experiments five male and five female broiler-type chicks (Peterson X Peterson) were randomly assigned to each of three replicate groups per treatment. A total of 23 dietary treatments were fed for a 21-day period. Routine brooding and evaluation procedures were as described in Chapter 2. On the final day of each experiment four birds in each replicate group (two of each sex) were sacrificed for tibia ash determinations.

Three basal diets were evaluated in each experiment. The composition of these diets is presented in Table 7. Diets 1, 2 and 3 contained 0, 3.0 and 6.0 percent fish meal, respectively. Diet 1 contained 0.41 percent phosphorus and 0.12 percent calcium. Diet 2 contained 0.48 percent phosphorus and 0.28 percent calcium. Diet 3 contained 0.57 percent phosphorus and 0.44 percent calcium. All three diets were formulated to be iso-caloric and iso-nitrogeneous. Each contained 22.07 percent protein and 2920 kilocalories of metabolizable energy per kilogram of feed. A total of 2200 I.C.U. of vitamin D_3 per kilogram of diet as well as other recommended level of micro-ingredients were supplemented.

Table 8 outlines the dietary treatments evaluated. Supplemental phosphorus was supplied from commercial grade soft phosphate (9.70 percent phosphorus and 18.24 percent calcium) and calcium phosphate (21.69 percent phosphorus and 20.92 percent calcium). Desired calcium levels were obtained by adding reagent grade calcium carbonate.

One level of soft phosphate and two levels of calcium phosphate were fed to chicksreceiving each basal diet. From one to four calcium levels were evaluated within each phosphorus source and level. Soft

Table 7. Composition of basal diets.

Ingredients	Diet 1 (%)	Diet 2 (%)	Diet 3 (%)
Yellow corn meal	53.71	55.17	56.48
Soybean meal (50% protein)	34.50	30.57	26.63
Alfalfa meal (20% protein)	3.00	3.00	3.00
Vegetable fat	1.71	1.25	0.90
Fish meal	----	3.00	6.00
Iodized salt	0.40	0.40	0.40
Micro-ingredients[1]	0.50	0.50	0.50
Methionine	0.18	0.11	0.09
Variable[2]	6.00	6.00	6.00
% phosphorus	0.41	0.48	0.57
% calcium	0.12	0.28	0.44

[1]Supplied per kilogram of diet: vitamin A, 6,600 I.U.; vitamin D_3, 2,200 I.C.U.; riboflavin, 4.4 mg.; pantothenic acid, 13.2 mg.; niacin, 39.6 mg.; choline chloride, 499.4 mg.; vitamin B_{12}, 22 mcg.; ethoxyquin, 125 mg.; manganese, 60 mg.; iron, 50 mg.; copper, 6 mg.; cobalt, 0.198 mg.; iodine, 1.1 mg.; zinc, 35 mcg.

[2]Included phosphate source, reagent grade calcium carbonate and builders' sand.

Table 8. Tibia ash and body weights of chicks fed various levels of phosphorus and calcium supplied by soft phosphate and calcium phosphate in practical corn-soybean type broiler diets with and without fish meal

Supplemental Phosphorus Source	Suppl. P (%)	Total P (%)	Total Ca (%)	Tibia ash[1] (%)	Body weights[1] (gms)
Diet 1, corn-soybean					
Soft phosphate	0.50	0.91	1.22	44.2^{abc}	358^{ab}
0.10% calcium phosphate & 0.40% soft phosphate	0.50	0.91	0.98	45.2^{bcdefg}	$373^{abcdefg}$
			1.18	45.6^{cdefgh}	371^{abcdef}
			1.38	44.1^{ab}	367^{abcde}
0.20% calcium phosphate & 0.30% soft phosphate	0.50	0.91	0.98	44.5^{bcd}	384^{efgh}
			1.18	46.9^{hij}	381^{defg}
			1.38	47.4^{j}	385^{efgh}
Calcium phosphate	0.36	0.77	0.90	44.8^{bcde}	392^{ghi}
	0.50	0.91	1.22	46.3^{fghij}	403^{hi}
Diet 2, corn-soybean + 3% fish meal					
Soft phosphate	0.40	0.88	1.04	45.2^{bcdefg}	360^{abc}
			1.18	45.4^{bcdefg}	359^{ab}
			1.33	44.3^{abc}	359^{ab}
			1.48	43.1^{a}	354^{a}
Calcium phosphate	0.26	0.74	0.71	44.2^{abc}	410^{i}
	0.40	0.88	1.04	46.5^{ghij}	380^{cdefg}
			1.18	45.9^{defghi}	380^{cdefg}
Diet 3, corn-soybean + 6% fish meal					
Soft phosphate	0.30	0.86	1.01	46.1^{efghij}	376^{bcdefg}
			1.14	47.0^{hij}	388^{fgh}
			1.29	47.3^{ij}	381^{defg}
			1.44	45.0^{bcdef}	361^{abcd}
Calcium phosphate	0.16	0.72	0.71	45.0^{bcdef}	376^{bcdefg}
	0.30	0.82	1.04	46.6^{ghij}	385^{efgh}
			1.18	47.0^{hij}	382^{efg}

[1]Means with different superscripts are significantly different according to Duncan's multiple range test ($P < 0.05$).

phosphate was supplemented to bring the phosphorus level to 0.91, 0.88 and 0.86 percent in diets 1, 2 and 3, respectively. Calcium phosphate treatments were supplemented to the same level as soft phosphate in each basal except for the 0.82 percent level in the 6.0 percent fish meal diet. Additional calcium phosphate treatments were supplemented to 0.77, 0.74 and 0.72 percent phosphorus in the three basal diets, respectively. Single calcium assignments were selected from standard curves proposed by Damron (1968). When a phosphorus treatment had more than one calcium level, these levels were selected to encompass the standard curve levels suggested by Damron (1968). Two corn-soybean dietary treatments contained phosphorus supplementation from combinations of soft and calcium phosphate sources. One received 0.10 percent phosphorus from calcium phosphate and 0.40 percent from soft phosphate for a supplemental total of 0.50 percent and a grand total of 0.91 percent phosphorus. The other combined phosphorus treatment received 0.20 percent phosphorus from calcium phosphate and 0.30 percent from soft phosphate for a supplemental total of 0.50 percent phosphorus. These treatments were designed to substitute calcium phosphate at those phosphorus levels supplied by the two fish meal diets.

Since both experiments were identical and statistical evaluation of the data revealed no significant treatment X experiment nor treatment X sex interaction, the experiments and sexes were combined for discussion.

Results and Discussion

The addition of 3.0 percent fish meal to the diet containing soft phosphate resulted in a numerical but not statistically significant increase in tibia ash (Table 8). These findings agree with those of

Damron et al. (1967); however, values observed in their studies numerically favored treatments without fish meal. When calcium: phosphorus ratios were optimum the addition of 6.0 percent fish meal to the diet containing soft phosphate resulted in a statistically significant increase in bone ash over the two other basal diets. Maximum bone ash was obtained with the combination of 6.0 percent fish meal and soft phosphate. The same level of bone ash was also obtained with a combination calcium phosphate - soft phosphate diet without fish meal.

The addition of 3.0 percent fish meal did not improve body weights of chicks receiving soft phosphate supplemented diets. These findings are not in agreement with Damron et al. (1967) who found a significant advantage in body weight for those four-week old chicks fed the soft phosphate supplemented diet with fish meal. However, addition of 6.0 percent fish meal to the diet containing soft phosphate yielded a significant increase in body weight over the chicks which received 0 and 3.0 percent fish meal when the calcium:phosphorus ratio was optimum. A deviation from this optimum ratio resulted in no differences among the three soft phosphate supplemented diets.

The addition of 3.0 or 6.0 percent fish meal to calcium phosphate supplemented diets did not improve bone ash nor body weight. Body weights, with only one exception, numerically favored the diet which contained no fish meal.

Two levels of phosphorus from calcium phosphate were observed within each basal diet. There appeared to be a slight trend toward a slight numerical advantage in bone ash when the additional 0.14 percent phosphorus was added in each of the three basal diets. However,

there was no difference in body weights under these same conditions except that the lower level of supplemental phosphorus significantly outperformed the 0.88 percent level in the 3.0 percent fish meal diet. This group had a lowered bone ash which may have allowed for a higher body weight.

Calcium phosphate consistently outperformed soft phosphate as measured by bone ash and body weight in the basal diets containing 0 and 3.0 percent fish meal. There were no differences, however, in the performance of soft phosphate and calcium phosphate as evaluated by either criterion when the 6.0 percent fish meal diet was fed. These data indicate that maximum performance can be obtained with soft phosphate when fed with high levels of fish meal. High levels of fish meal tended to improve the performance of soft phosphate diets, but did not improve the performance of calcium phosphate diets. Substitution of 0.1 and 0.2 percent phosphorus from calcium phosphate for the two levels of fish meal resulted in a linear improvement in performance comparable to that obtained with the fish meal. This suggests that phosphorus in fish meal is equal to the phosphorus in calcium phosphate under these conditions. There appeared to be no unidentified factor benefit from fish meal since chicks receiving diets containing calcium phosphate performed equally as well, if not superior to, those containing fish meal as a source of a comparable amount of phosphorus.

Calcium:phosphorus ratios in these experiments were critical, but high levels of fish meal tended to minimize the effects of these ratios in soft phosphate supplemented treatments. Ratios appeared to be less critical when calcium phosphate was the phosphorus source. These

findings agree with previous findings reviewed in this dissertation.

It was noted that optimum performance was obtained when soft phosphate supplied no more than 0.3 percent of the phosphorus in the diet. It was demonstrated that maximum performance cannot be obtained when 0.4 or 0.5 percent levels of phosphorus from soft phosphate are the only supplemental source. Previous work reported in this dissertation agrees with this point, and also agrees with Johnson et al. (1953) who recommended that no more than 0.2 percent phosphorus be supplied from soft phosphate.

Most of the treatments in these experiments contained approximately 0.9 percent phosphorus. This level is considerably more than the National Research Council recommendations (1971). Treatments with approximately 0.76 percent phosphorus, for the most part, performed equally as well as those with higher levels.

Summary

Two experiments involving 23 treatments were conducted with broiler-type chicks to evaluate the utilization of soft phosphate and calcium phosphate in practical corn-soybean type diets with and without fish meal. Two levels of fish meal (3.0 and 6.0 percent) were evaluated. Comparable phosphorus and calcium levels were formulated in each of the three basal diets. Phosphorus supplementation was reduced to compensate for the phosphorus content of the fish meal.

The addition of 3.0 or 6.0 percent fish meal numerically improved bone ash in soft phosphate supplemented diets. There was no improvement in body weight with 3.0 percent added fish meal,

but 6.0 percent added fish meal gave an increase in body weight over diets containing 0 and 3.0 percent fish meal. Whether the improvement in soft phosphate utilization with added fish meal is due to a more available phosphorus source is not known. Calcium phosphate supplemented diets outperformed soft phosphate diets when fed with the 0 or the 3.0 percent fish meal diet. There was no difference in the performance of the chicks fed two inorganic phosphorus sources in the 6.0 percent fish meal diet. Maximum performance was obtained with soft phosphate in combination with 6.0 percent fish meal. Maximum performance was also obtained with soft phosphate when phosphorus in fish meal was substituted with an equal amount from calcium phosphate. The level of fish meal in the diet did not affect bone ash of calcium phosphate supplemented diets when ratios were comparable.

Maximum bone ash was not obtained with calcium phosphate alone or in combination with fish meal. Why maximum bone ash could be obtained with soft phosphate in combination with high levels of fish meal or calcium phosphate is not clearly understood.

Phosphorus from fish meal performed equally as well as did that from calcium phosphate when substituted at comparable levels. The addition of fish meal to the diet gave no increased advantages that could not be obtained when calcium phosphate supplied a comparable amount of phosphorus.

CHAPTER 7

Summary

Years of extensive investigation on the availability of phosphorus in various materials have produced a vast amount of knowledge about the subject. However, much of the research reported has been quite variable, and has uncovered many additional unanswered questions. For example, there is a need to determine the type of assay diet best suited for the study of phosphorus availability. Diets tried have varied from purified to practical, with most researchers selecting one of many in between. Considerable research has been conducted to determine the calcium level or calcium ratio which would give maximum response for each phosphorus source.

No uniformly acceptable response measurement has been developed to determine relative phosphorus availabilities. However, in recent years, percent bone ash and growth rate have become the accepted primary criteria used by most investigators. Even these criteria have yielded different results with several phosphorus materials. The phosphorus in plant sources has been found to be quite available when body weight was the evaluation criterion, but not so available when bone ash served as the response measurement. In contrast, soft phosphate has been found to be quite available for mineralization but not very available for growth. These findings indicate that different forms of phosphorus may perform specific metabolic roles for body processes. Considerable data suggest that the feeding of various

combinations of sources, both organic and inorganic, might be beneficial.

The main thrust of the studies reported in this dissertation was: 1) to determine the influence of various types of diets in the assay of phosphorus materials, primarily soft phosphate, and 2) to determine possible metabolic complementary effects of combinations of phosphorus sources.

A series of trials involving broiler-type chicks or Single Comb White Leghorn cockerels was conducted to determine if purified diets could be utilized to assay soft phosphate, if there were specific metabolic functions for the phosphorus found in soft phosphate and plant phosphate and if combinations of soft phosphate with other phosphorus materials could complement or enhance the value of soft phosphate. Except for phosphorus and calcium levels, six different basal diets were formulated to meet accepted nutrient requirements for the chick. All trials were of two or three weeks duration. Chicks were housed in wire floored, electrically heated, battery-type brooders. Tibia ash, body weight and mortality rate were the evaluation criteria. All data were tabulated and subjected to analysis of variance.

One series of experiments involving Single Comb White Leghorn cockerels was conducted to determine the usefulness of a purified diet in soft phosphate analysis. Mortality was extremely high, but not statistically different regardless of treatment. The addition of soft phosphate, generally, did not improve bone ash. The addition of sodium acid phosphate gave a numerical but not statistically significant improvement in bone ash. Furnishing calcium phytate phosphorus in addition to the other supplements did not improve bone ash or

livability. Due to the high mortality, this diet did not prove to be ideal for the assay of phosphorus sources under the conditions of these experiments.

Two experiments were conducted to determine whether plant phosphorus and soft phosphate were selectively available to the chicks for specific functions and would complement each other when fed in combination. A hominy feed basal diet with 0.50 percent phosphorus and a degerminated corn meal diet with 0.30 percent phosphorus were employed in these studies. The results of these trials confirmed that soft phosphate might be quite available for bone mineralization but not for growth. A linear response was obtained in tibia ash and body weight from feeding supplemental levels of 0.07, 0.14 and 0.21 percent phosphorus from either soft phosphate or calcium phosphate when degerminated corn meal was utilized. Chicks fed the diet with the low level of soft phosphate had bone ash levels numerically superior to those obtained with calcium phosphate. A linear response in tibia ash was also obtained from adding each level of calcium phosphate to the hominy feed diet. An increase in bone ash was obtained from supplementing this diet with soft phosphate, but it was not linear except when calcium:phosphorus ratios were increased. It would appear that a hominy feed diet may be useful in phosphorus assays since growth rate is equal for all groups containing supplemental phosphorus, thus removing this factor from consideration in phosphorus availability. It appears that under certain conditions soft phosphate phosphorus and plant phosphorus might be complementary in obtaining body weight and bone ash.

In the third series, calcium:phosphorus ratios were confirmed as being very important in phosphorus availability. It was also determined that the less available the phosphorus was in the supplement the more critical was the calcium:phosphorus ratio. In hominy feed diets these ratios became less critical as optimum phosphorus levels were approached. Hominy diets containing 0.50 percent phosphorus, all from plant sources, yielded good growth but poor bone ash. Little difference was noted in body weight from increasing phosphorus levels as long as calcium:phosphorus ratios were comparable. A graduated increase in phosphorus resulted in a numerically linear increase in bone ash regardless of the source. Again this suggested that more emphasis can be placed on bone ash and less on body weight when hominy feed diets are employed for phosphorus assay purposes. Lower levels of phosphorus from soft phosphate appeared to be more available for bone ash than were comparable levels from calcium phosphate. As soft phosphate levels increased, the availability decreased. Although soft phosphate was not statistically inferior, calcium phosphate consistently gave better performance. Calcium:phosphorus ratios and calcium levels appeared to be more critical for soft phosphate utilization than for calcium phosphate utilization.

A practical corn-soybean meal type diet was used for the fourth series of studies reported in this dissertation. The purpose of these trials was to determine the feasibility of combining soft phosphate with fish meal, as well as to determine if the phosphorus in fish meal could be replaced by an available phosphorus source without the loss of diet performance. The addition of 3.0 or 6.0 percent fish meal

improved bone ash in corn-soybean type diets. There was no improvement in body weight with the addition of 3.0 percent fish meal; however, the addition of 6.0 percent fish meal improved body weight over that obtained with 0 and 3.0 percent fish meal. When the phosphorus in fish meal was replaced with an equal amount from calcium phosphate, performance was not changed. These results indicated that there was no unknown factor in fish meal in addition to the phosphorus it supplied. Maximum body weights and bone ash were obtained with soft phosphate and 6.0 percent fish meal. Maximum performance could also be obtained with the soft phosphate when some phosphorus was supplied from calcium phosphate.

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BIOGRAPHICAL SKETCH

Robert Bruce Christmas was born October 31, 1933, near Cottondale, Florida. He graduated from Cottondale High School in 1951, serving as president of the student body during his senior year. He attended Chipola Junior College for two years and was elected to the honor society and office of the vice president of the student body. He received the Bachelor of Science in Agriculture degree with honors from the University of Florida in 1955. As an undergraduate, he was elected to Alpha Zeta, Gamma Sigma Delta and Phi Kappa Phi.

After serving two years as a communications specialist in the United States Army, he was admitted to the graduate school of the University of Florida and received the Master of Science in Agriculture degree in 1959. While pursuing his master's degree he was the author or co-author of eight publications. For the next seven years he served as an assistant and associate county agricultural agent in Orlando, Fla., with work emphasis in the area of livestock and field crop programs.

He returned to the University of Florida in 1967 to pursue the Ph.D. degree in Poultry Nutrition. He served as part-time assistant to the Provost of Agriculture during his two-year campus study period.

He became supervisor of the Florida National Egg Laying Test in 1969. He is an active member of the Baptist Church and is active in civic affairs. He is married to the former Addie Ann Cartledge of Cottondale, Florida, and they have four sons, Stuart, Bruce Jr., Jon and Scott.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert H. Harms, Chairman
Professor of Poultry Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Clarence B. Ammerman
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Tony J. Cunha
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Shaw E. Grigsby
Professor of Extension Administration

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

H R Wilson
Henry R. Wilson
Associate Professor of Poultry Science

This dissertation was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

March, 1972

John E. Reynolds
Asst Dean, College of Agriculture

Dean, Graduate School